The Amendment

Independent Claims 9, 27, and 28 have been amended to more clearly recite that the sperm being treated is intact and viable. Support for this limitation is found on page 6, lines 12-23, of the specification.

The Rejection Under 35 USC 103

Claims 9-34 stand rejected under 35 USC 103 as being unpatentable over Johnson et al. (*Gamete Research* 17), Johnson et al. (*Gamete Research* 16) and Johnson et al. (*Gamete Research* 21) all in view of Dresser et al. (GB 2,145,112) and *Handbook of Histopathological and Histochemical Techniques*. This is the singular rejection in the case, and its withdrawal is requested for the reasons which follow.

The Johnson et al. publications are all drawn to the separation of sperm heads (nuclei) only which have been treated in some fashion to sever the tails and midsections from the heads. In Johnson et al. (*GR*16), chinchilla sperm were minced, filtered and twice centrifuged prior to staining (paragraph bridging pages 2 and 3). It is clear from the discussion in the publication and from the illustration in Figure 1 that only the sperm nuclei are stained and sorted.

Johnson et al. (*GR*17) collects bull semen and subjects it to various treatments to include sonication or solvent fixation (see pages 204-205 and FIG. 1). The sonication has the effect of severing the tails and midsections from the sperm heads and the solvent

fixation denatures the cell protein. At the bottom of page 209, it is stated that the several preparation procedures described in the paper were evaluated for the purpose of determining the minimal treatment conditions necessary to resolve the X and Y chromosome-bearing sperm populations while maintaining DNA integrity. However, there is no mention of an attempt to preserve the integrity of the sperm morphology (*i.e.*, to prevent severing of the sperm tails from the heads). At the top of page 211, it is indicated that sonication was a quick and efficient method for destroying membrane integity and allowing penetration of the stain. The top of page 211 also states that sonication is less destructive morphologically to the sperm than treatment with papain/DTE, suggesting that significant cell disruption would also be imparted by the latter treatment.

Johnson et al. (*GR*21) treats semen from bulls, boars, and rams by extending in saline, centrifuging, sonicating, and again centrifuging. After staining, the resultant sperm nuclei are used for microinjection (bottom page 3).

In each of the Johnson et al. publications, the staining is conducted on tailess sperm heads at room temperature (25° C). Though the staining temperature may not be specifically recited, it is generally understood in scientific publications that a procedure is conducted at room temperature unless otherwise stated. Submitted herewith is a

declaration by applicant Johnson, who was a coauthor on each of the cited publications. Johnson attests that the staining procedures of sperm heads described in each of the applied publications were indeed conducted on tailess sperm at room temperature. As noted by the Examiner, the time period for the staining treatment was 30 min. in each case. This was a standard time used by the authors for the sake of uniformity. However, a much shorter time (*e.g.*, 15 min.) would have been satisfactory for the dead sperm heads, inasmuch as stain penetrates easily after membrane breakage.

The various treatments described in the Johnson publications for disrupting the cell membranes in order to facilitate staining at room temperature also renders them useless for fertilization when used in artificial insemination. Not only is the morphological integrity disrupted, but also the sperm cells are rendered nonviable. Mammalian sperm require motility (active metabolism) to enable them to swim to the site of fertilization, and then to penetrate the egg and fertilize it. Sperm heads are not capable of any part of the natural fertilization process if they do not possess the midpiece/tail complex which gives them that motility. Sperm must be in perfect condition to fertilize an egg. That is why under natural conditions, a vast overabundance of sperm are ejaculated for the fertilization of a single egg.

Applicant acknowledges that the Johnson et al. publications teach that sperm heads can be stained and separated using a cell sorter as described in the present application. However, given the state of the art at the time of filing the application, there are several reasons why it could not be construed from the references that the sorting procedure therein would be effective for intact and viable sperm.

Firstly, it cannot be projected from the teachings in the references that intact and viable sperm can be sufficiently stained to permit sorting. As established in the response and Declaration Under 37 CFR 1.132 submitted on May 30, 1991, staining of live sperm cells at room temperature requires about 3 or more hours. Upon incubating sperm under those conditions, the preponderance of the population is no longer viable and would not be suitable for fertilization. Submitted herewith are Declarations Under 37 CFR 1.132 by three experts in the art (Duane L. Garner, Dan Pinkel, and Charles H. Allen) attesting to this fact. These experts also attest to the general attitude in the art regarding the inadvisability of handling sperm at elevated temperatures prior to artificial insemination. They further state that the various treatments preparatory to staining the sperm cells in each of the publications would in every case cause the tails and midsections of the sperm to become severed from the sperm heads and that the membranes surrounding the resultant sperm heads would thereby be disrupted.

Secondly, there is nothing in the applied art to suggest that the cell sorting system of the Johnson et al. publications would be effective to sort intact sperm, that is, sperm having tails. The living mammalian sperm is 80 μ long, the tail accounting for 70 μ of that length (see Fig. 3, "Diagrammatic representation of a spermatozoan" in *The Biochemistry of Semen*, enclosed). Tailess sperm (ovoid shapes 10 μ long) are relatively passive and are therefore readily susceptible to orientation and manipulation in a cell sorter. In contrast, the interaction between the hydrodynamic forces of the sorter and the intrinsic motility of a viable sperm cell with a flagellating tail lend a significant level of unpredictability to the orientation and sorting process. Absent the applicant's disclosure, it would be impossible to predict that the sorting method and device of the publications would sort intact and viable sperm.

Thirdly, it would not be intuitively obvious that the sperm would remain viable and intact upon passing through the cell sorter. Examples 2-4 and the data in Tables I and II of applicant's disclosure demonstrate that rabbit and swine sperm sorted in accordance with the invention are capable of fertilization when used in artificial insemination.

The Examiner has relied upon the *Handbook of Histopathological and Histochemical Techniques* for the general teaching that "[a] reduction in staining time of certain procedures may usually be effected by the application of heat." The Examiner has taken the position that it would

be obvious from this teaching to incubate sperm within the claimed range of 30-39° C in order to shorten the staining time of the Johnson et al. publications, in the absence of objective evidence to the contrary. In general, this combination of teachings is considered to be inappropriate in that there is no suggestion in Johnson et al. that higher staining temperatures could be employed, and there is no suggestion in the handbook that the described techniques could be applied to sperm cells. The technique discussed in the handbook relates to cells being prepared for mounting. These cells are typically nonliving and are often in some way disrupted. Moreover, there is no attempt to preserve viability of cells being mounted for histopathological or histochemical purposes. Except at the very low end of the 37-56° C range indicated in the reference, sperm cells would immediately be killed. As discussed above, applicants have successfully stained living cells without substantially affecting their viability. This is considered to be objective evidence in support of an unexpected result.

The combination of the Johnson et al. publications with the Handbook also fails to suggest that the degree of staining which can be achieved at the claimed temperature range and claimed time period is sufficient to obtain a bimodal separation of the living sperm in a cell sorter. The person in the art will appreciate that the staining of a cell would cover an entire spectrum. Even assuming that the skilled artisan would be led by the combination of references to try to stain living sperm within the

skilled artisan would be led by the combination of references to try to stain living sperm within the claimed temperature and time ranges, there is no basis for predicting that the stained product would be sortable. The claimed process requires a delicate balance of conditions which will both preserve viability and permit sorting of intact sperm.

The Examiner has again raised the issue that it would be obvious to incubate sperm with the claimed temperature range because sperm exist *in situ* in the testes and vagina at these temperatures. As discussed in applicant's Preliminary Response dated May 30, 1991 (pages 1 and 2), the protocol for handling semen *ex situ* is to maintain it below room temperature for short periods of time and at subzero temperatures for extended periods. These temperatures have been established in the trade of artificial insemination as those being optimal for preserving sperm viability, notwithstanding the well-established scientific knowledge about the physiological conditions of spermatogenesis and fertilization. In considering who is a person in the art of handling sperm *ex situ*, one would necessarily consider those in the trade of artificial insemination. Accordingly, applicant submits that it would not have been obvious to a person in the art to ignore the standard protocols for handling sperm *ex situ* in favor of irrelevant physiological conditions.

It is unclear why the Examiner has relied upon the GB patent, other than for its teaching of staining sperm for 2 hours at room temperature. This teaching is not considered to be any more relevant than those of the Johnson et al. publications. The arguments presented above regarding the Johnson et al. publications are deemed to apply to the GB patent.

Summary

The claims have been amended to clarify that the sperm being treated in accordance with the invention are intact and viable. The staining process must be sufficient to not only permit sorting into X- and Y-chomosome-bearing populations, but also to preserve the sperm viability. Applicants have demonstrated why the combination of references fail to suggest staining sperm within the claimed temperature range for achieving this dual goal. Insofar as the Johnson et al. publications deal with tailess sperm having disrupted membranes which are easily stained at room temperature and which are easily sorted in a cell sorter, they fail to provide a predictable basis for the claimed invention. The modification of the Johnson et al. process urged by the Examiner in view of the *Handbook of Histopathological and Histochemical Techniques* is contrary to the established protocols in the art of artificial insemination.

Applicant submits that he has met his burden of overcoming the *prima facie* case of obviousness advanced by the patent Examiner. Accordingly, all the claims submitted for examination are believed to distinguish over the art of record and allowance thereof is earnestly solicited.

Respectfully submitted,

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6 Enclosure:
Johnson Declaration Under 37 CFR 1.132
Garner Declaration Under 37 CFR 1.132 w/Curriculum Vitae
Pinkel Declaration Under 37 CFR 1.132 w/Curriculum Vitae
Allen Declaration Under 37 CFR 1.132 w/Curriculum Vitae
Replacement copy of reference R: *Handbook of Histopathological and Histochemical Techniques*
FIG. 3, "Diagrammatic representation of a spermatozoan," in *The Biochemistry of Semen*